AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-14 (CANCELED)

15. (CURRENTLY AMENDED) A composition comprising:

a pharmaceutically acceptable carrier, diluent or excipient; and at least one non-virulent strain of bacteria produced by the process comprising:

introducing at least one mutation into the genome of a bacteria;

culturing the mutated bacteria in the presence of an antimicrobial agent ~~that~~ for a time and at a concentration of the antimicrobial that effectively kills growing but ~~not~~ has reduced affect on or does not kill non-growing bacteria;

selecting surviving bacteria;

testing the selected surviving bacteria for virulence;

and selecting the non-virulent strains.

16. (ORIGINAL) The composition of claim 15, wherein said bacteria is a mycobacteria.

17. (ORIGINAL) The composition of claim 16, wherein said bacteria is a slow growing mycobacteria.

18. (ORIGINAL) The composition of claim 17, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis.*

19. (ORIGINAL) The composition of claim 15, wherein said mutation is by insertion of a transposon.

20. (ORIGINAL) The composition of claim 15, wherein said mutation is a random mutation.

21. (ORIGINAL) The composition of claim 15, wherein said antimicrobial agent is a fluoroquinolone.

22. (ORIGINAL) The composition of claim 21, wherein said fluoroquinolone is Bay y 3118.

23. (PREVIOUSLY AMENDED) The composition of claim 22, wherein said Bay y 3118 is used at a concentration of at least 0.015 μg/mL.

24. (ORIGINAL) The composition of claim 15, wherein said antimicrobial is D-cycloserine.

25. (ORIGINAL) The composition of claim 24, wherein D-cycloserine is used at a concentration of at least 25 μg/mL.

26. (ORIGINAL) The composition of claim 15, wherein said mutated bacteria is cultured in an intracellular culture system.

27. (ORIGINAL) The composition of claim 26, wherein said intracellular culture system is a macrophage culture system.

28. (CURRENTLY AMENDED) A composition comprising:

a pharmaceutically acceptable carrier, diluent or excipient;

and at least one non-virulent strain of *M. paratuberculosis* produced by the process comprising:

introducing at least one random mutation into the genome of a strain of *M. paratuberculosis* by insertion of a transposon;

infecting macrophages with the mutated strain;

culturing the infected macrophages in the presence of a fluoroquinolone or D-cycloserine; for a time and at a concentration that effectively kills growing but has reduced affect on or does not kill non-growing bacteria;

selecting surviving *M. paratuberculosis* organisms;

testing the selected surviving organisms for virulence in an animal; and

selecting the non-virulent strains.

29. (CURRENTLY AMENDED) A composition comprising:

a pharmaceutically acceptable carrier diluent or excipient;

and at least one bacterial virulence determinant, the determinant identified by a process comprising;

introducing at least one mutation into the genome of a bacteria;

culturing the mutated bacteria in the presence of an antimicrobial agent ~~that~~ for a time and at a concentration of antimicrobial that effectively kills growing but ~~not~~ has reduced affect on or does not kill non-growing bacteria;

selecting surviving bacteria;

testing the selected surviving bacteria for virulence;

selecting the non-virulent strains;

sequencing genetic material from the selected non-virulent bacteria to determine the site of the mutation; and

identifying the virulence determinant based on the site of the mutation.

30. (ORIGINAL) The composition of claim 29, wherein said bacteria is a mycobacteria.

31. (ORIGINAL) The composition of claim 30, wherein said mycobacteria is a slow growing mycobacteria.

32. (ORIGINAL) The composition of claim 31, wherein said slow growing mycobacteria is *Mycobacterium paratuberculosis.*

33. (ORIGINAL) The composition of claim 29, wherein said mutation is by insertion of a transposon.

34. (ORIGINAL) The composition of claim 29, wherein said mutation is a random mutation.

35. (ORIGINAL) The composition of claim 29, wherein said antimicrobial agent is a fluoroquinolone.

36. (ORIGINAL) The composition of claim 35, wherein said fluoroquinolone is Bay y 3118.

37. (ORIGINAL) The composition of claim 36, wherein said Bay y 3118 is used at a concentration of at least 0.015 μg/mL.

38. (ORIGINAL) The composition of claim 29, wherein the antimicrobial is D-cycloserine

39. (ORIGINAL) The composition of claim 38, wherein said D-cycloserine is used at a concentration of at least 25 μg/mL.

40. (ORIGINAL) The composition of claim 29, wherein said mutated bacteria is cultured in an intracellular culture system.

41. (ORIGINAL) The composition of claim 40, wherein said intracellular culture system is a macrophage culture system.

42. (CURRENTLY AMENDED) A composition comprising:

a pharmaceutically acceptable carrier diluent or excipient;

and at least one *Mycobacterium paratuberculosis* virulence determinant, the determinant identified by a process comprising;

introducing at least one mutation into the genome of a strain of *Mycobacterium paratuberculosis* by insertion of a transposon;

infecting macrophages with the mutated strain;

culturing the infected macrophages in the presence of a fluoroquinolone or D-cycloserine; for a time and at a concentration that effectively kills growing but has reduced affect on or does not kill non-growing bacteria;

selecting surviving bacteria;

testing the selected surviving bacteria for virulence in an animal; selecting the non-virulent bacteria;

sequencing genetic material from the selected non-virulent bacteria to determine the site of the mutation; and

determining the virulence determinant based on the site of the mutation.

Claims 43-53 (CANCELED)

54. (NEW) The composition of Claim 15, wherein said antimicrobial agent is a quinolone.